GENETIC MARKERS WHICH IDENTIFY INDIVIDUALS WHO IMPROVE THEIR DIABETES STATUS WITH EXERCISE

FIELD OF THE INVENTION

The present invention relates to identifying one or more genetic markers which correlate with greater success in improving diabetes status in individuals with and without diabetes.

BACKGROUND OF THE INVENTION

Studies have shown that individuals suffering from or at risk of developing diabetes can alleviate symptoms or otherwise improve their conditions through exercise. Unfortunately, some individuals, no matter how rigorously they exercise, are unable to improve their conditions, while others benefit to a much greater extent than predicted. These results underscore the fact that many factors contribute to an individual's well-being. Such factors include, for example, behaviors such as diet and exercise, genetic makeup, and environment. While behavior and environment can be controlled, altered or regulated, an individual's genetic makeup is essentially predetermined and set at birth. The present inventors hypothesized that upon identifying the genetic makeup of a population suffering from or at risk of developing diabetes and observing that some individuals of the population improve their diabetic status from a change of behavior to a much greater or lesser extent than expected, a correlation could be made between the presence or absence of certain genetic markers and success in improving diabetic status.

An object of the present invention is to identify one or more genetic markers which positively correlate with greater success in improving diabetes status in individuals with and without diabetes.

SUMMARY OF THE INVENTION

The present inventors have discovered a number of genetic markers which positively correlate with greater success in improving diabetes status in

diabetic or at risk individuals, as compared with other genetic makeup at the same gene loci. Therefore, the present invention is directed to a method of improving diabetes status in a subject with diabetes or at risk of developing diabetes, the method comprising:

identifying a subject with diabetes or at risk of developing diabetes having an allele and/or a genotype at a gene locus which positively correlates with greater success in improving diabetes status in diabetic individuals, as compared with other alleles and/or genotypes at the same gene locus; and

engaging the subject in exercise training for a period of time sufficient to improve the diabetes status in the subject.

DETAILED DESCRIPTION OF THE INVENTION

The inventors have found that a number of genetic markers positively correlate with greater success in improving diabetes status in individuals with diabetes or at risk of developing diabetes, as compared with other genetic makeup at the same gene loci. Markers which the inventors have investigated include the beta-2 and beta-3 adrenergic system receptor (ASR) gene, the peroxisome proliferator activator receptor gamma (PPAR-gamma) gene, the insulin receptor substrate-1 (IRS-1) gene and the fatty acid binding protein-2 (FABP-2) gene.

The term "subject in need of improvement" means both subjects with diabetes and subjects at risk of developing diabetes. In a preferred embodiment, the subject is with diabetes.

The term "improved diabetes status" means an improvement in at least one characteristic area which is associated with diabetes. An improvement may be in one or more of the following characteristic areas (this list is non-exhaustive and includes overlapping and representative examples only): change in glucose metabolism, change in insulin metabolism, change in glucose levels from a baseline determination, change in insulin levels from a baseline determination, change in fasting plasma glucose levels, change in fasting

plasma insulin levels or change in acute insulin response to glucose. These improvements may be measured by, for example, glucose tolerance tests conducted before and after exercise training. An improvement in diabetes status in accordance with the invention may be found both in individuals with diabetes and in individuals at risk of developing diabetes.

The term "single course of exercise", as used throughout this application, means a cardiovascular exercise session of any type which is conducted during one day. An exercise session may comprise an aerobics class, treadmill training, step machine, or any other suitable cardiovascular exercise regimen. For most cases, exercise may be completed in, for example, 30 minutes to 3 hours, with optional brief rest periods of 3-15 minutes, however this amount would vary depending on the health and endurance of the subject.

The term "moderate exercise" means about 5-9 single courses of exercise, preferably about 6-8, or 7 single courses of exercise, over the exercise period. The exercise period in the case of a moderate exercise protocol may be from about 5-45 days, preferably from about 5-30 days, 5-20 days, or 5-15 days.

The term "extensive exercise" means about 10 single courses of exercise or more, preferably at least 15, at least 20, or at least 25 single courses of exercise, over a defined period of time ("the exercise period"). The exercise period in the case of an extensive exercise protocol may be from about 50-400 days, preferably from about 70-350 days or 100-300 days.

The time between exercise courses depends on whether the exercise period is an extensive or moderate exercise period. In the case of extensive exercise periods, the time between exercise courses may be from about 1-3 days or more. In the case of moderate exercise periods, the time between exercise courses may be from 24 hours or more.

The present inventors have discovered that diabetic individuals or those at risk of developing diabetes with different genotypes for genes which control the manufacture of certain proteins exhibit different degrees of success in

improving their diabetes status through exercise. The inventors have surprisingly discovered that each genotype potentially can benefit from exercise, however, the amount of exercise which produces the most benefits varies according to genotype. These results could not have been predicted from initial patient screening.

Beta adrenergic receptors have numerous functions in the body, and are activated by binding beta agonists, such as the catecholamines isoproterenol, norepinephrine and epinephrine. In mammals, the liberation of glucose and fatty acids can be triggered by the binding of epinephrine or norepinephrine to beta adrenergic receptors on hepatic and adipose cells.

The inventors have found that subjects having a "12" genotype for a beta-2 adrenergic receptor gene exhibit a greater improvement in diabetes status than those with a "11" genotype, after extensive exercise. However, subjects having the "11" genotype for the beta-2 adrenergic receptor gene exhibit a greater improvement in diabetes status than those with the "12" genotype, after moderate exercise.

Therefore, one method of improving diabetes status in a subject in need of such improvement according to the invention comprises identifying a subject having a "12" genotype for a beta-2 adrenergic receptor gene, wherein the subject is in need of improved diabetes status and engaging the subject in extensive exercise training for a period of time sufficient to improve the diabetes status in the subject.

Another method of improving diabetes status in a subject in need of such improvement according to the invention comprises identifying a subject having a "11" genotype for a beta-2 adrenergic receptor gene, wherein the subject is in need of improved diabetes status and engaging the subject in moderate exercise training for a period of time sufficient to improve the diabetes status in the subject.

The present inventors have also discovered that diabetic individuals with different beta-3 adrenergic receptor genotypes exhibit different degrees of

success in improving their diabetes status through exercise. The inventors have found that those individuals having a "11" genotype exhibit greater improvement in diabetes status than those with "12" genotypes, after extensive exercise.

Therefore, an additional method of improving diabetes status in a subject in need of such improvement according to the invention comprises identifying a subject having a "11" genotype for a beta-3 adrenergic receptor gene, wherein the subject is in need of improved diabetes status and engaging the subject in extensive exercise training for a period of time sufficient to improve the diabetes status in the subject.

Peroxisome proliferator activator receptors are members of the nuclear hormone receptor family of transcription factors, a diverse group of proteins that mediate ligand-dependent transcriptional activation and repression. They modulate DNA transcription by binding to specific peroxisome proliferator response elements on target genes. The best characterized of these receptors, PPAR-gamma, is known to play a critical role in adipocyte differentiation and fat deposition and is highly expressed in this tissue.

The inventors have found that diabetics or those at risk of developing diabetes having a "11" genotype at the PPAR-gamma locus improve their diabetes status more with extensive exercise training than those having a "12" genotype.

Therefore, a method of improving diabetes status in a subject in need of such improvement comprises identifying a subject having a "11" genotype for a PPAR-gamma gene, wherein the subject is in need of improved diabetes status and engaging the subject in extensive exercise training for a period of time sufficient to improve the diabetes status in the subject.

IRS-1 is a 185 kDa protein which is activated rapidly upon insulin stimulation of cells, and is a key mediator of an insulin-regulated biological activity. The amino-terminal region of the protein contains interaction modules that facilitate its binding to receptors of insulin, IGF-1 and others. The

remainder of the molecule contains numerous tyrosine containing motifs, which, when phosphorylated by the insulin receptor tyrosine kinase, serve as binding regions for a variety of cellular proteins containing a so-called "SH2" domain.

The inventors have found that subjects having a "12" genotype for the IRS-1 gene exhibit a greater improvement in diabetes status than those with a "11" genotype, after extensive exercise.

Therefore, in accordance with this aspect of the present invention, a method of improving diabetes status in a subject in need of such improvement comprises identifying a subject having a "12" genotype for an IRS-1 gene, wherein the subject is in need of improved diabetes status and engaging the subject in extensive exercise training for a period of time sufficient to improve the diabetes status in the subject.

Fatty acid binding protein (FABP) can be found in numerous places in the body. In the liver, this protein binds free fatty acids and their Coenzyme A derivatives, bilirubin, and some other small molecules in the cytoplasm. Intestinal FABP is an abundant cytosolic protein in small intestine epithelial cells. It may participate in the uptake, intracellular metabolism and/or transport of long-chain fatty acids.

The inventors have found that subjects having a "12" genotype for a FABP-2 gene exhibit a greater improvement in diabetes status than those with a "11" genotype, after extensive exercise.

Therefore, a method of improving diabetes status in a subject in need of such improvement, according to this aspect of the invention, comprises identifying a subject having a "12" genotype for a FABP-2 gene, wherein the subject is in need of improved diabetes status and engaging the subject in extensive exercise training for a period of time sufficient to improve the diabetes status in the subject.

EXAMPLES

Example 1. Variations in Improvement of Diabetes Status in Subjects with Different Beta-2 and Beta-3 ASR and PPAR-Gamma Genotypes After Extensive Exercise

DNA was obtained from obese sedentary men 50-65 yrs of age, and processed as follows.

Detection of Pro12Ala Substitution in PPAR-Gamma

Genotyping for the Pro12Ala substitution in PPARγ2 was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis as previously reported (1). Briefly, genomic DNA (approximately 20 ng) was subjected to PCR using upstream primer 5'-GCCAATTCAAGCCCAGTC-3' and mutagenic downstream primer 5' -GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG-5' using standard reagents and cycling conditions to yield a 270 bp product. If the C→G substitution at nucleotide 34 of the PPARγ2 gene is present, the mutagenic downstream primer introduces a BstU-1 restriction site (CG/CG). Digestion with BstU-1 was performed; the products were electrophoresed on a 2.5% agarose gel, the gel was stained with ethidium bromide, and DNA was visualized by UV transillumination. Expected digestion product sizes were 270 bp for Pro12 homozygotes, 227 and 43 bp for Ala12 homozygotes, and 270 bp, 227 bp, and 43 bp for heterozygotes.

Detection of Trp64Arg Substitution in Beta-3-ASR

Polymerase chain reaction was performed from approximately 20 ng of genomic DNA with upstream primer 5'-CGCCCAATACCGCCAACAC-3' and downstream primer 5'-CCACCAGGAGTCCCATCACC-3' in the presence of 10% dimethylsulfoxide (2). The resulting 210 bp product was digested with BST NI. The digested products were subjected to electrophoresis through a 4% agarose gel. The gel was stained with ethidium bromide and DNA was

visualized by UV transillumination. The expected sizes were 99 bp, 62 bp, 30 bp, 12 bp and 7 bp for Trp64 homozygotes, 161 bp, 30 bp, 12 bp and 7 bp for Arg64 homozygotes, and 161 bp, 99 bp, 62 bp, 30 bp, 12 bp and 7 bp for heterozygotes.

Detection of Gln27Glu Substitution in Beta-2 ASR

Genotyping for the Gln27Glu substitution in the beta-2-adrenergic receptor was performed as described previously (3). Genomic DNA was amplified with upstream primer 5'-GGCCCATGACCAGATCAGCA-3' and downstream primer 5'-GAATGAGGCTTCCAGGCGTC-3' using standard conditions. The resulting 353 bp product was digested with Ita I and the reaction products subjected to agarose gel electrophoresis. The gel was stained with ethidium bromide and DNA was visualized by UV transillumination. The expected sizes were 174 bp, 97 bp, 55 bp and 27 bp for Gln27 homozygotes, 229 bp, 97 bp and 27 bp for Glu27 homozygotes, and 229 bp, 174 bp, 97 bp, 55 bp and 27 bp for heterozygotes.

Results

The subjects underwent 9 months of endurance exercise training to quantify, among other things, their improvements in plasma glucose and insulin responses to an oral glucose challenge. Subjects were initially stabilized on an American Heart Association low-fat diet and then underwent an oral glucose tolerance test with blood samples drawn for up to 3 hours after the ingestion of a standard glucose load. This diet was maintained throughout the 9 months of exercise training and subjects repeated the glucose tolerance test after training. The data in Table 1 represent the change in the integrated glucose and insulin areas above baseline that occurred with exercise training. Subjects with the beta-2 ASR "12" genotype decreased their glucose and insulin areas with exercise training substantially more than subjects with the beta-2 ASR "11" genotype. Furthermore, subjects with the beta-3 ASR "11" genotype decreased

their glucose areas less, but decreased their insulin areas substantially more with exercise training than subjects with the beta-3 ASR "12" genotype. Lastly, subjects with the PPAR-gamma "11" genotype decreased their glucose areas less, but decreased their insulin areas dramatically more with exercise training than subjects with the PPAR-gamma "12" genotype. Thus, these results indicate that beta-2 and beta-3 ASR and PPAR-gamma genotypes identify those individuals most likely to improve their diabetic status, in terms of glucose and insulin metabolism, with exercise training.

Table 1: Change with Exercise Training in Integrated Glucose and Insulin Areas in Response to an Oral Glucose Tolerance Test as a Function of Genotype

	Change with Exercise Training	
β2 Receptor Genotype	**Glucose Area**	**Insulin Area**
11 genotype (n=6)	-285 ± 791	-3224 ± 1584
12 genotype (n=8)	-1489 ± 603	-6831 ± 5349
β3 Receptor Genotype		
11 genotype (n=13)	-1012 ± 559	-6863 ± 3930
12 genotype (n=2)	-2115 ± 495	-1273 ± 1192
PPARγ Genotype		
11 genotype (n=13)	-1255 ± 536	-9150 ± 4308
12 genotype (n=2)	-2692 ± 472	172 ± 891

Values are mean ± SE. Values are expressed as the change with 9 months of exercise training in integrated glucose or insulin area above baseline for 3 hours following a standard oral glucose challenge. Thus, negative values indicate a response that is reduced after exercise training and positive values a response that is greater after training.

Example 2. Variations in Improvement of Diabetes Status in Subjects with Different IRS-1 and FABP-2 Genotypes After Extensive Exercise

The same subjects as in Example 1 were genotyped for IRS-1 and FABP-2 genes as follows.

Detection of Gly972Arg Substitution in IRS-1

A 220 bp region encompassing the Gly972Arg substitution was amplified from approximately 20 ng of genomic DNA with upstream primer 5'-GCAGCCTGGCAGGAGAGCCAT-3' and downstream primer 5'-CTCACCTCCTCTGCAGCAATG-3'. PCR products were digested with Bst NI. The digested products were run on a 4% agarose gel, stained with ethidium bromide, and visualized by UV transillumination. The expected digestion product sizes were 220 bp for Gly972 homozygotes, 164 bp and 56 bp for Arg972 homozygotes and 220 bp, 164 bp and 56 bp for heterozygotes.

Detection of Ala54Thr Substitution in FABP-2

A 180 bp region encompassing the Ala54Thr substitution was amplified by PCR from genomic DNA with upstream primer 5'-ACAGGTGTTAATATAGTGAAAAG-3' and downstream primer 5'-TACCCTGAGTTCAGTTCCGTC-3' using standard conditions (4). The PCR product was digested with Hha I. The digestion products were electophoresed through a 4% agarose gel, the gel was stained with ethidium bromide, and DNA was visualized by UV transillumination. Expected DNA fragment sizes were 99 bp and 81 bp for Ala54 homozygotes, 180 bp for Thr54 homozygotes and 180 bp, 99 bp and 81 bp for heterozygotes.

Results

The exercise regimen for these subjects was described in Example 1. The data in the following Table 2 represent the change in the integrated glucose and insulin areas above baseline and fasting plasma insulin levels that occurred

with the exercise training. Subjects with the FABP-2 "12" genotype decreased their glucose and insulin areas and fasting insulin levels with exercise training substantially more than subjects with the FABP-2 "11" genotype. Furthermore, subjects with the IRS-1 "12" genotype decreased their glucose areas somewhat less, but decreased their insulin areas and fasting insulin levels substantially more with exercise training than subjects with the IRS-1 "11" genotype. Thus, these results indicate that IRS- 1 and FABP-2 genotypes identify those individuals most likely to improve their diabetic status, in terms of glucose and insulin metabolism, with exercise training.

Table 2: Change with Exercise Training in Integrated Glucose and Insulin Areas in Response to an Oral Glucose Tolerance Test and Fasting Plasma Insulin Levels as a Function of Genotype

	Change with Exercise Training		
FABP-2 Genotype	**Glucose Area**	**Insulin Area**	**Fasting Insulin**
11 genotype (n=6)	-221 ± 674	-2320 ± 1021	-1.6 ± 2.2
12 genotype (n=5)	-2232 ± 509	-10080 ± 8559	-7.5 ± 3.7
IRS-1 Genotype			
11 genotype (n=9)	-1437 ± 490	-2308 ± 854	-2.6 ± 2.0
12 genotype (n=2)	-49 ± 1588	-21771 ± 22230	-11.7± 7.1

Values are mean ± SE. Values are expressed as the change with 9 months of exercise training in integrated glucose or insulin area above baseline for 3 hours following a standard oral glucose challenge or the fasting plasma insulin level. Negative values indicate a response or level that is reduced after exercise training and positive values a response that is greater after training.

Example 3. Variations in Improvement of Diabetes Status in Subjects with Different Beta-2 ASR Genotypes After Moderate Exercise

In the study obese sedentary hypertensive African-American middle-aged women underwent 7 days of endurance exercise training to quantify, among other things, their improvements in plasma glucose and insulin responses to an intravenous glucose challenge. DNA processing was in accordance with Example 1. Subjects were initially weight-stabilized on their own diet and then underwent an intravenous glucose tolerance test with blood samples drawn for up to 3 hours after the injection of a standard glucose load. The women then repeated the glucose tolerance test after 7 days of exercise training. With training, women with the beta-2 ASR "11" genotype decreased their acute insulin response, during the first 10 minutes following the injection of the glucose load, and their fasting plasma insulin levels more than women with the beta-2 ASR "12" genotype. The results are presented in the following Table 3. These results are further evidence that the beta-2 ASR genotype identifies those individuals most likely to improve their diabetic status, in terms of glucose and insulin metabolism, with exercise training.

Table 3: Change with Moderate Exercise Training in Acute Insulin Response and Fasting Insulin Levels as a Function of Genotype

	Change with Exercise Training	
β2 Receptor Genotype	**Acute Insulin Response**	**Fasting Insulin**
11 genotype (n=5)	-36 ± 64	0.2 ± 1.5
12 genotype (n=3)	-169 ± 201 (P=0.20)	-5.7 ± 3.1 (P=0.009)

Values are mean ± SD. Values are expressed as the change with 7 days of exercise training in acute insulin response for the 10 minutes following the injection of glucose and fasting plasma insulin levels. Negative values indicate a response or level that is reduced after 7 days of exercise training and positive

values a response or level that is greater after training.

The following references cited in the specification are hereby incorporated by reference:

(1) Yen et al. (1997) *Biochem. Biophys. Res. Commun.* **241**, 270-274.

(2) Widen et al. (1995) *N. Engl. J. Med.* **333**, 348-351.

(3) Large et al. (1997) *J. Clin. Invest.* **100**, 3005-3013.

(4) Baier et al. (1995) *J. Clin. Invest.* **95**, 1281-1287.